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Claims

1. A method of determining the area or confluency of a sample, comprising:
  - 5 providing quantitative phase data relating to the sample and background surrounding the sample;  
determining from the quantitative phase data the boundary of the sample; and  
determining the area within the boundary in order to  
10 determine either the area of the sample or the confluency of the sample.
2. The method of claim 1 wherein the quantitative phase data is obtained by detecting light from the sample by a  
15 detector so as to produce differently focused images of the sample, and determining from the different images the quantitative phase data by an algorithm which solves the transport of intensity equation so as to produce a phase map of the sample in which the phase data is contained.
- 20 3. The method of claim 1 wherein the step of determining the boundary of the sample comprises forming a histogram of quantitative phase data measurements of the sample and background, taking the derivative of the histogram to  
25 thereby determine the point of maximum slope of the histogram in the vicinity of the boundary of the sample, and determining a line of best fit on the derivative to obtain a data value applicable to the boundary so that data values either above or below the determined data  
30 value are deemed within the sample.
4. The method of claim 3 wherein the step of determining the area or confluency comprises determining the area of confluency from the number of data samples which are  
35 within the boundary.

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5        5.    The method of claim 4 wherein each data sample is applicable to a pixel of a detector and the area of each pixel is known, so that from the known area of the pixels and the number of pixels which register a data value above or below the predetermined data value, the area or confluency of the sample is determined.

6.    A method of determining the area or confluency of a sample comprising:  
10        detecting light emanating from the sample by a detector to form at least two images of the sample which are differently focused to provide two sets of raw data; from the two sets of raw data, determining a quantitative phase map of the sample and its background;  
15        determining a boundary of the sample from individual phase data values applicable to pixels of the detector which are either above or below a determined pixel value; and  
      determining the area or confluency by multiplying the  
20        pixel area by the number of pixels which are either above or below the determined pixel value to thereby determine the area or confluency of the sample.

7.    The method of claim 6 wherein the pixel values are  
25        grey scale values and grey scale values above a determined grey scale value are deemed to be within the sample and are multiplied by the pixel area to determine the area or confluency of the sample.

8.    The method of claim 6 wherein the determined pixel  
30        value is determined by identifying the greatest rate of change of grey scale pixel values, thereby identifying the boundary of the sample.

9.    The method of claim 8 wherein the greatest rate of  
35        change is determined by forming a histogram of grey scale values for all of the pixels which detect the sample and

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- its background, determining the derivative of the histogram to provide a graphical measure of the greatest rate of change of grey scale values at various pixels, and determining the line of best fit of the curve to determine the grey scale value which defines the boundary of the sample so that all grey scale values which are greater than the determined grey scale value are deemed to be within the sample.
- 10 10. The method of claim 6 wherein the raw data comprises at least one in focus image of the sample and at least one out of focus image of the sample.
- 15 11. The method of claim 10 wherein the raw data comprises the in focus image of the sample and one positively defocused image and one negatively defocused image of the sample.
- 20 12. An apparatus for determining the area or confluency of a sample, comprising:  
a processor for:  
receiving quantitative phase data relating to the sample and background surrounding the sample;  
determining from the quantitative phase data the  
25 boundary of the sample; and  
determining the area within the boundary in order to determine either the area of the sample or the confluency of the sample.
- 30 13. The apparatus of claim 12 wherein the apparatus further comprises a detector for producing differently focused images of the sample, and the processor is for determining from the different images the quantitative phase data by an algorithm which solves the transport of  
35 intensity equation so as to produce a phase map of the sample in which the phase data is contained.

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14. The apparatus of claim 13 wherein the processor determines the boundary of the sample by forming a histogram of quantitative phase data measurements of the sample and background, taking the derivative of the histogram to thereby determine the point of maximum slope of the histogram in the vicinity of the boundary of the sample, and determining a line of best fit on the derivative to obtain a data value applicable to the boundary so that data values either above or below the determined data value are deemed within the sample.
15. The apparatus of claim 13 wherein the processor determines the area or confluency by determining the area of confluency from the number of data samples which are within the boundary.
16. The apparatus of claim 15 wherein each data sample is applicable to a pixel of a detector and the area of each pixel is known, so that the processor, from the known area of the pixels and the number of pixels which register a data value above or below the predetermined data value, determines the area or confluency of the sample.
17. An apparatus for determining the area or confluency of a sample comprising:  
a detector for detecting light emanating from the sample to form at least two images of the sample which are differently focused to provide two sets of raw data;  
a processor for determining from the two sets of raw data, a quantitative phase map of the sample and its background;  
the processor also determining a boundary of the sample from individual phase data values applicable to pixels of the detector which are either above or below a determined pixel value; and  
the processor also determining the area or confluency by multiplying the pixel area by the number of pixels

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which are either above or below the determined pixel value to thereby determine the area or confluency of the sample.

18. The apparatus of claim 17 wherein the pixel values are grey scale values and grey scale values above a determined grey scale value are deemed to be within the sample and are multiplied by the pixel area to determine the area or confluency of the sample.
19. The apparatus of claim 18 wherein the determined pixel value is determined by identifying the greatest rate of change of grey scale pixel values, thereby identifying the boundary of the sample.
20. The apparatus of claim 19 wherein the greatest rate of change is determined by the processor forming a histogram of grey scale values for all of the pixels which detect the sample and its background, determining the derivative of the histogram to provide a graphical measure of the greatest rate of change of grey scale values at various pixels, and determining the line of best fit of the curve to determine the grey scale value which defines the boundary of the sample so that all grey scale values which are greater than the determined grey scale value are deemed to be within the sample.
21. The apparatus of claim 17 wherein the raw data comprises at least two defocused images equally spaced either side of the focus.
22. The apparatus of claim 21 wherein the raw data comprises the in focus image of the sample and one positively defocused image and one negatively defocused image of the sample.
23. A computer program for determining the area or confluency of a sample from providing quantitative phase

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data relating to the sample and background surrounding the sample, comprising:

code for determining from the quantitative phase data the boundary of the sample; and

- 5       code for determining the area within the boundary in order to determine either the area of the sample or the confluency of the sample.

24. The computer program of claim 23 wherein the  
10       quantitative phase data is obtained by detecting light from the sample by a detector so as to produce differently focused images of the sample, and the program includes code for determining from the different images the  
      quantitative phase data by an algorithm which solves the  
15       transport of intensity equation so as to produce a phase map of the sample in which the phase data is contained.

25. The computer program of claim 23 wherein the code for determining the boundary of the sample comprises code for  
20       forming a histogram of quantitative phase data measurements of the sample and background, code for taking the derivative of the histogram to thereby determine the point of maximum slope of the histogram in the vicinity of the boundary of the sample, and code for determining a  
25       line of best fit on the derivative to obtain a data value applicable to the boundary so that data values either above or below the determined data value are deemed within the sample.

- 30       26. The computer program of claim 23 wherein the code for determining the area or confluency comprises code for determining the area of confluency from the number of data samples which are within the boundary.

- 35       27. The computer program of claim 26 wherein each data sample is applicable to a pixel of a detector and the area of each pixel is known, so that from the known area of the

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pixels and the number of pixels which register a data value above or below the predetermined data value, the area or confluency of the sample is determined.

- 5 28. A computer program for determining the area or confluency of a sample by detecting light emanating from the sample by a detector to form at least two images of the sample which are differently focused to provide two sets of raw data, comprising:
- 10       code for determining from the two sets of raw data, a quantitative phase map of the sample and its background;  
          code for determining a boundary of the sample from individual phase data values applicable to pixels of the detector which are either above or below a determined
- 15 pixel value; and  
          code for determining the area or confluency by multiplying the pixel area by the number of pixels which are either above or below the determined pixel value to thereby determine the area or confluency of the sample.
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29. The computer program of claim 28 wherein the pixel values are grey scale values and grey scale values above a determined grey scale value are deemed to be within the sample and are multiplied by the pixel area to determine
- 25 the area or confluency of the sample.
30. The computer program of claim 28 wherein the determined pixel value is determined by code for identifying the greatest rate of change of grey scale
- 30 pixel values, thereby identifying the boundary of the sample.
31. The computer program of claim 30 wherein the greatest rate of change is determined by code for forming a
- 35 histogram of grey scale values for all of the pixels which detect the sample and its background, code for determining the derivative of the histogram to provide a graphical

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measure of the greatest rate of change of grey scale values at various pixels, and code for determining the line of best fit of the curve to determine the grey scale value which defines the boundary of the sample so that all  
5 grey scale values which are greater than the determined grey scale value are deemed to be within the sample.

32. The computer program of claim 28 wherein the raw data comprises at least one in focus image of the sample and at  
10 least one out of focus image of the sample.

33. The computer program of claim 32 wherein the raw data comprises the in focus image of the sample and one positively defocused image and one negatively defocused  
15 image of the sample.